Biochimica et Biophysica Acta, 603 (1980) 228—236

BBA 79014

BLOCK OF SODIUM CONDUCTANCE BY *n*-OCTANOL IN CRAYFISH GIANT AXONS

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(Received April 21st, 1980)
(Revised manuscript received June 30th, 1980)

Key words: Na^+ current; Octanol; Voltage clamp; (Giant axon)

Summary

The block of the Na^+ current by *n*-octanol was studied in crayfish giant axons under axial wire voltage-clamp conditions. Standard kinetic analysis of the Na^+ currents was undertaken to test the hypothesis that the *n*-octanol-induced block of the Na^+ current could be accounted for on the basis of changes in the voltage dependence of the kinetic parameters. Alterations in the membrane dipolar potential arising from rearrangement of membrane lipids would be the anticipated source of changes in the voltage dependence. Although some changes in voltage dependence did evolve with the block by *n*-octanol, the changes were not of sufficient magnitude to account for the block. In conclusion, although higher concentrations of *n*-octanol produced shifts along the voltage axis of the kinetic parameters, direct blocking action of *n*-octanol on the channel appears to be the most important mechanism of the block.

Introduction

Anesthetic molecules have been found to produce a variety of alterations of membrane structure, and these observations have led to several hypotheses explaining the molecular mechanism of anesthesia. Among the most notable membrane changes used to develop theories of anesthesia are membrane expansion and fluidization.

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A variety of measurements suggest that the membrane expands during anesthesia [1—4]. The observed expansion of red blood cell membranes [2] and model membrane systems has led to two discrete theories of anesthesia, corresponding to a proteinaceous or lipid site. Anesthetics may directly induce conformational changes in membrane proteins rendering them inactive [5,6]. Alternatively, those favoring a lipid site suggest that expansion of the membrane lipids may mechanically squeeze ionic channels closed [7—10].

Anesthetics also cause fluidization and disordering of membrane components as shown by NMR measurements in natural [11] and artificial membranes [10]. The combination of this information and measurements of ion concentration changes in cerebral spinal fluid [12] and permeability changes in axonal membranes [13] have led others to suspect these anesthetic-induced alterations as the key to anesthesia. The accumulation of Na^+ inside small axons of the brain following increased resting Na^+ permeability could produce anesthesia.

A third manner in which anesthetic molecules alter membrane structure is through the membrane field [14]. *n*-Octanol and benzocaine were found to decrease the surface dipole potential in phosphatidylcholine bilayer membranes by 125 and 93 mV, respectively. The authors argued in support of the premise that anesthetics exert their primary influence on ionophores, not by alterations in fluidity but rather by altering the dipolar component of the membrane surface potential. Therefore, anesthetics might be expected to radically alter the voltage dependence of conductance parameters which determine thresholds of excitability.

We decided to test the latter hypothesis for the action of *n*-octanol on crayfish giant axons. If the 'electrostatic model' of anesthesia is correct, we would expect that the block of the Na^+ current caused by *n*-octanol [15] should be accounted for by shifts in the voltage dependence of the Hodgkin-Huxley parameters, m_∞ and τ_m, in the direction of depolarization and/or h_∞ and τ_h in the direction of hyperpolarization. Moreover, to substantiate this hypothesis, these alterations would have to be large enough in magnitude to account for all the observed decrease in the Na^+ current at all membrane potentials, without requiring any direct block of the Na^+ channel or decrease in $\bar{g}_{Na}$.

Methods

The crayfish, *Procambarus clarki*, were purchased and maintained in tap water at 8—12°C. The nerve chamber was made of Teflon-coated aluminum with a rectangular lucite insert with a raised central platform. An extension of the aluminum through which solutions passed was used as a cooling block on which a thermoelectric cooling device was fixed. Temperature was maintained at 11, 12, or 13°C in all experiments. The axon was laid in the lucite-aluminum chamber with the dorsal side up, and the ends pinned down on either side with Teflon rollers, and illuminated from below with a fiber optics light.

A modified van Harreveld solution [16] was used as the bathing medium. To minimize series resistance problems, a solution with one-half the normal Na^+ concentration was used. Na^+ currents were generally of the order of 1 mA/cm^2. Cl^- concentration was also decreased in this solution to minimize the accumu-

lation of Cl^- inside the axon during the course of a long-term experiment. The modified solution consisted of 105 mM tetramethylammonium chloride, 105 mM sodium isethionate, 2.6 mM $MgSO_4$, 13.5 mM $CaCl_2$, 5 mM KCl and 3 mM Hepes buffer. The pH of the solution was titrated to 7.55 with tetramethylammonium hydroxide. The osmolarity of the solution was 460-480 mosmol per kg. 3,4-Diaminopyridine (0.5 mM) was added to the solution to block the K^+ currents.

Test solutions containing *n*-octanol were prepared from 1 M stock solution of *n*-octanol dissolved in dimethylsulfoxide (Me_2SO). An aliquot of the stock solution was injected into a rapidly spinning volume of the bathing solution. This technique allowed effective concentrations of *n*-octanol above the normal solubility to be tested. 1 mM *n*-octanol was completely soluble in the bathing solution, but the '10 mM' *n*-octanol solution was a suspension of small micelles. Thus, the 10 mM solution was considered to be saturated with octanol, as well as containing additional octanol available for binding.

The piggy-back-style axial electrode [17] was filled with a solution developed by Shrager [18] as an internal perfusate. This solution consisted of 247 mM potassium citrate and 18 mM NaCl titrated to pH 7.5 with citric acid. The reference electrode was composed of a glass Pasteur pipette with the end tapered and fire polished. The electrode was filled with a solution of modified van Harreveld's solution and 2% agar. The connection to the potential measuring circuit was made with an Ag/AgCl pellet molded around a piece of silver wire inserted in the agar. The external current and guard electrodes were made of a lucite block with three pieces of platinum foil glued to two surfaces. The two outside had a longitudinal dimension of 2 mm, while the central electrode (current-measuring electrode) was 1 mm in length. This electrode assembly was coated with a thin layer of platinum black using the Kohlrausch solution.

The standard voltage-clamp technique and circuit were used for these experiments. The circuit could achieve a rise time of 8—12 μs for a voltage pulse of 100 mV. The current-measuring amplifier had a response time of less than 1 μs. The leakage and capacitative components of the membrane current were subtracted directly using a transient subtraction bridge.

Series resistance (R_s) was measured by passing a step current pulse with a rise time of less than 1 μs as described by Cole and Moore [19]. However, this technique allowed only an estimate of the upper limit of R_s of 1—2 $\Omega \cdot cm^2$. As overcompensation of R_s will lead to the same error as uncompensated R_s, no compensation was attempted. Rather, the maximum errors due to a change in the membrane current through R_s were calculated. These computations suggested that the problem was not significant for interpreting the results, and are described in Results.

Steady-state inactivation (h_∞) was measured using the standard double-pulse technique of Hodgkin and Huxley [20]. A long conditioning pulse (25 ms) to a series of membrane potentials was followed by a test pulse to −25 mV. The peak amplitude of the Na^+ current was measured from the film records. The data were plotted as the ratio of the peak current to the maximum peak current as a function of the conditioning membrane potential.

The time constants for Na^+ activation (τ_m) and inactivation (τ_h) and the

steady-state Na^+ activation (m_∞) were measured by plotting an Na^+ current on a logarithmic scale as a function of time. The falling phase of the Na^+ current expressed a single exponential function from which τ_h was directly determined by extrapolating back to time zero ($\bar{g}_{Na} m_\infty^3 h_0$). The time required for this product to reach 0.37 of its value is equivalent to τ_h. The value of τ_m was measured from this plot according to the method of Hille [21]. The value of m_∞ could also be calculated from this plot. The point of intersection ($\bar{g}_{Na} m_\infty^3 h_0$) of the straight line with the y-axis yielded the initial current at time zero (I'_{Na}). At time zero, h becomes unity, and therefore by dividing I'_{Na} by the driving force for Na^+, g'_{Na} results. By calculating the relative g'_{Na} (i.e., $g'_{Na}/g'_{Na_{max}}$), m_∞ can be derived according to the equation:

$$m_\infty = \sqrt[3]{g'_{Na}/g'_{Na_{max}}}$$

Results

Continuous application of n-octanol at concentrations as low as 6.25 μM to intact crayfish giant axons decreased the Na^+ current slowly and eventually blocked it completely. Recovery from complete block after washing was very slow or almost absent. However, when application of octanol was stopped at intermediate levels of block, the Na^+ current recovered slowly and eventually attained the control level. Since it took a long time for the Na^+ current to decrease to a level suitable for measurements (about 50% of the control) by continuous application of a low concentration of octanol, measurements were made during the slow washout phase after brief application of high concentrations (1 or 10 mM) of octanol. If the measurements at the end of the experiment were different from that shortly after starting washout, the experimental data were discarded. It should be emphasized that the measurements of Na^+ conductance parameters made under these conditions must represent steady-state effects of a concentration of octanol lower than 6.25 μM which eventually blocks Na^+ current completely. In the text, the observed effects are referred to the actions of 1 or 10 mM octanol for the sake of convenience, but

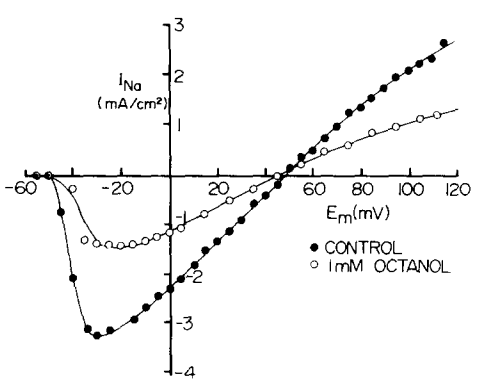

Fig. 1. The relationship between the peak Na^+ current and the membrane potential before and after treatment with 1 mM octanol in an intact crayfish giant axon.

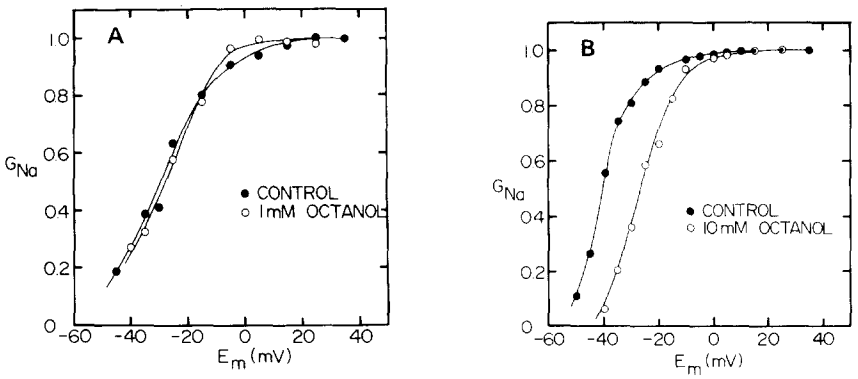

Fig. 2. The normalized Na^+ conductance plotted as a function of the membrane potential in voltage-clamped crayfish giant axons. A, before and after treatment with 1 mM octanol (the mean of six experiments). The average block of the current by octanol was 54%. B, before and after treatment with 10 mM octanol (the mean of four experiments). The average block of the Na^+ current was 59%.

such statements by no means indicate the steady-state effects of octanol at these two concentrations.

Fig. 1 shows the current-voltage relationships for Na^+ current before and after application of 1 mM octanol. Both inward and outward Na^+ currents were blocked approx. 50%. The Na^+-reversal potential (E_{Na}) was unchanged by the alcohol.

Na^+ activation system

The 'turning-on' process of crayfish axon membranes was altered by octanol in the manner predicted by the electrostatic model as described previously. Small depolarizing voltage pulses which were large enough to elicit Na^+ currents in control axons frequently did not produce current in the presence of octanol. As a first step to examine changes in the activation system, the relationship between the conductance and the membrane potential was measured before and after application of 1 and 10 mM octanol. Fig. 2 shows the pooled results of several experiments. Na^+ conductance (g_{Na}), normalized to its maximum value, was plotted as a function of the membrane potential (E_m). The g_{Na}-E_m curve was not appreciably shifted by 1 mM octanol (Fig. 2A), but shifted by 14 mV in the direction of depolarization by 10 mM octanol (Fig. 2B). However, the average block of g_{Na} by the two concentrations of octanol was almost the same, being 54% by 1 mM (six experiments) and 59% by 10 mM (four experiments). This suggests two separate effects of octanol, one blocking and the other shifting the voltage dependence of g_{Na}. A shift by 3—4 mV in the depolarizing direction would be expected due to the error caused by the maximum series resistance of $2\ \Omega \cdot cm^2$. Moreover, as the block and the decrease of Na^+ current were roughly the same in these two sets of experiments, the shift caused by uncompensated series resistance should also be roughly equivalent.

Time constant of activation

The time required for the Na^+ current to reach its peak (time-to-peak) and the Na^+ activation time constant, τ_m, were prolonged by octanol. The pooled

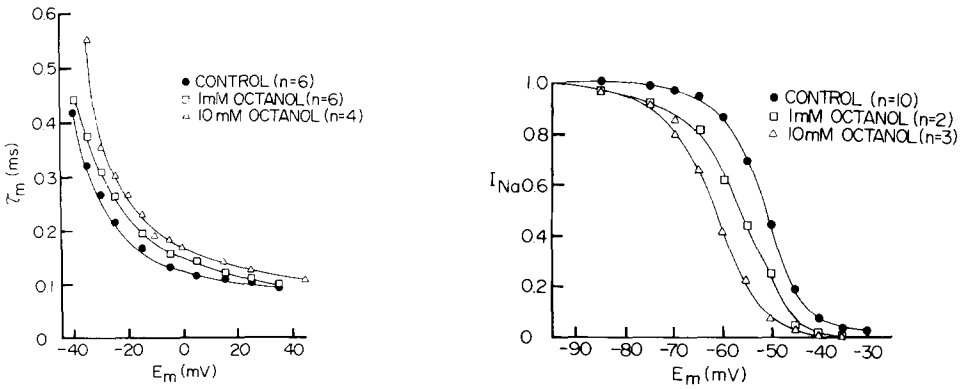

Fig. 3. Time constant of Na^+ activation (τ_m) plotted as a function of the membrane potential before and after treatment with 1 and 10 mM octanol.

Fig. 4. Steady-state Na^+ inactivation curves before and after treatment with 1 and 10 mM octanol. The holding potential was —75 mV. The half-points of the hand-drawn curves are —50, —56 and —63 mV for control, 1 and 10 mM octanol, respectively.

results of τ_m measurements as a function of the membrane potential in both 1 and 10 mM octanol are shown in Fig. 3. The values of τ_m obtained in 1 mM octanol were not significantly different from those of the control at the 0.05 level, whereas all the 10 mM points were significantly different from the controls. Detailed computer simulations of the influence of uncompensated series resistance revealed that no measurable change would be expected at potentials more positive than —20 mV, with R_s as high as 6 $\Omega \cdot cm^2$.

Steady-state inactivation

In control experiments it was found that the steady-state Na^+ inactivation (h_∞) curve was identical when the membrane was held at —75, —85 and —95 mV before applying conditioning and test pulses. Fig. 4 shows the steady-state inactivation curves before and after application of 1 and 10 mM octanol. Noticeably, the curve was shifted being approx. 4 mV in 1 mM octanol and 11 mV in 10 mM octanol. These shifts were not large enough to account for the observed decrease in Na^+ conductance. For example, the average block of the maximum inward Na^+ current by 1 mM octanol in the two experiments shown in Fig. 4 was approx. 40%, yet the h_∞ value at —75 mV decreased only 6%. This result was expected from the previous experiments in which long hyperpolarizing prepulses were found to relieve only partially the alcohol block.

Uncompensated series resistance would be expected to underestimate the shift of the inactivation curve along the voltage axis by the alcohol, but computations revealed no significant change in the curve in these experiments with R_s values up to 6 $\Omega \cdot cm^2$.

Time course of inactivation

Although 1 mM octanol had little detectable influence on the time constant of Na^+ inactivation measured from the falling phase of Na^+ current (Fig. 5A), a dramatic alteration was observed when 10 mM octanol was applied (Fig. 5B).

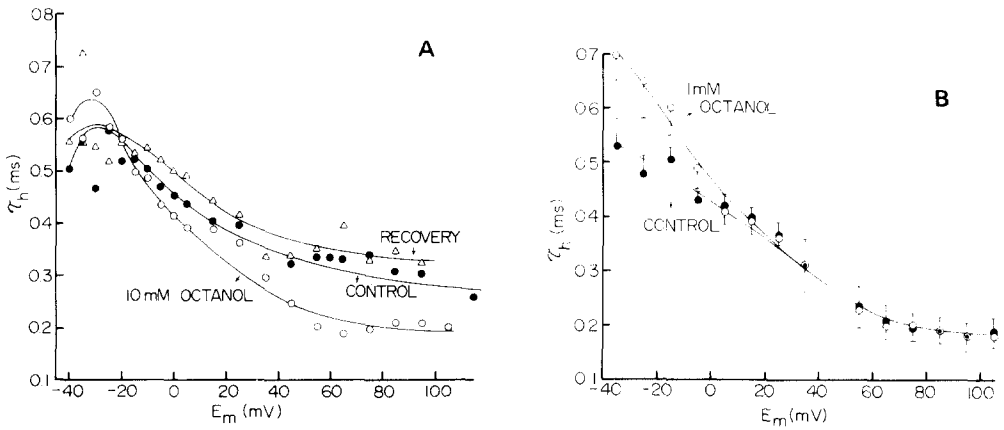

Fig. 5. Time constant of Na^+ inactivation (τ_h) plotted as a function of the membrane potential before (n = 6) and after (n = 6) treatment with 1 mM octanol (A), and before (n = 4) and after (n = 4) treatment with 10 mM octanol and after washing (n = 4) (B). The time constant of inactivation was measured from the falling phase of the Na^+ current. Vertical bars represent the standard deviations.

The control curve of τ_h vs. E_m was similar to that described by Hodgkin and Huxley [20] for squid axons, but the effect of octanol did not seem to be a simple suppression or voltage shift in the relation. This may have, at least in part, resulted from the difficulty of resolving the hump in the curve thought to exist at low depolarizations. Except at membrane potentials more negative than −20 mV, τ_h was speeded up by 10 mM octanol. A similar speeding up of τ_h by octanol has been observed in squid axons [22]. In one experiment in which 1 mM decanol was used, the same pattern was observed.

Although uncompensated series resistance would theoretically produce a similar alteration in the τ_h vs. E_m relationship, computations using R_s values up to 6 $\Omega \cdot cm^2$ revelaed a maximum change of only 8%, much smaller than that found at large depolarizations in 10 mM octanol.

Slow inactivation

Shrager [23] has shown that crayfish giant axons have a prevalent slow Na^+ inactivation system which is affected by sulfhydryl reagents. A long hyperpolarizing prepulse completely reversed the block of Na^+ current caused by *N*-ethylmaleimide [18]. Fig. 6 shows experiments in which the block of the Na^+ current by 1 mM octanol was diminished by holding the membrane potential at more negative values. This procedure serves to eliminate slow Na^+ inactivation present at the normal holding potential of −75 mV, and any additional inactivation produced by octanol. It was found that octanol shifts the curve relating the slow inactivation to the membrane potential as well as the fast inactivation in the hyperpolarized direction. Similar results have been found in squid giant axons [22]. However, the block could not be relieved completely by either applying more negative holding potential or long hyperpolarizing prepulses (squares in Fig. 6). In the prepulse experiment, the 49% Na^+ current block found with no prepulse at a holding potential of −75 mV was reduced to 18% by the prepulse to −115 mV.

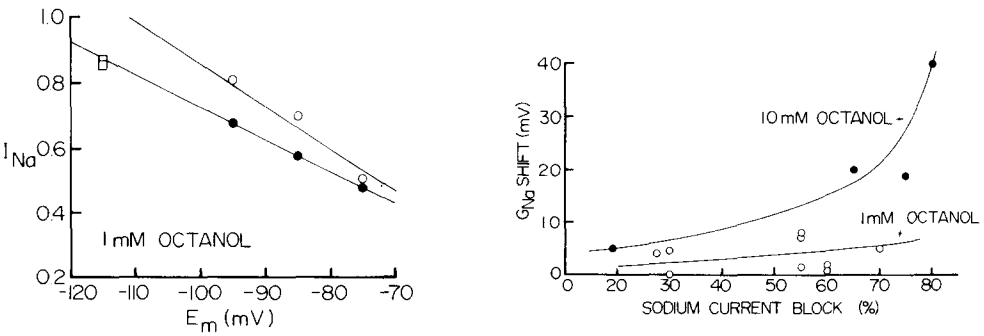

Fig. 6. Relief from the octanol (1 mM) block of the peak Na^+ current by hyperpolarizing holding potential (two experiments, ○ and ●) or by hyperpolarizing prepulse to −115 mV lasting 110 ms (one experiment, □).

Fig. 7. Relationship between the shift of Na^+ conductance along the voltage axis as measured at the 50% maximum conductance and the percentage of block of Na^+ conductance caused by 1 and 10 mM octanol.

Discussion

Alterations in the voltage dependence of Na^+ activation and inactivation parameters caused octanol have been found to be insufficient to account for the observed block of the Na^+ current. Although changes in the voltage dependence of τ_m, m_∞, τ_h and h_∞ by 1 mM octanol are in the direction postulated by the electrostatic model of anesthesia, they account for no more than 10% of the total block produced by octanol. The experiments with 10 mM octanol have revealed a second effect of octanol on crayfish axons. It appears that octanol at the high concentration can attain sufficiently high concentrations in the membrane to alter the voltage dependence of Na^+ conductance parameters by a means independent of its channel-blocking mechanism. This dualism can be easily seen in Fig. 7, where the shift of the g_{Na} along the membrane potential axis is plotted as a function of the percent block of the peak Na^+ current for 1 mM octanol (○) and 10 mM octanol (●). It is clear that at comparable block of the Na^+ current, 10 mM octanol causes a greater shift of the g_{Na} along the membrane potential axis. Therefore, we conclude that one of the major mechanisms by which octanol blocks Na^+ channels is either a decrease in the number of conducting channels or a decrease in the conductance of a single Na^+ channel. These two mechanisms cannot easily be discriminated without performing single-channel recording or fluctuation analysis. Recent experiments on the effect of octanol on the gating currents in squid giant axons support the notion that the channel-gating mechanism is affected [22,24]. The relationship between the 'on' charge movement and membrane potential is not shifted by octanol along the voltage axis [22].

The shift of the steady-state Na^+ inactivation in the hyperpolarizing direction along the voltage axis by octanol is in accordance with that found with several local anesthetics including benzocaine [25,26]. A decrease in the time constant of Na^+ inactivation has also been described for local anesthetics [27] and other blocking compounds [28]. The small depolarizing shift of m_∞ along the voltage axis was found to be within the limits of that arising from uncom-

pensated series resistance. The depolarizing shift of τ_m may at least in part be the result of a change in electric field across the membrane in the presence of octanol. A delay in the activation of the Na^+ current was observed when hyperpolarizing prepulses preceded a test pulse [29]. This would be opposite in direction to the situation for octanol.

Although experiments with artificial membranes [14] suggested that a change in the membrane field produced by octanol might account for the previously reported decrease in Na^+ conductance [15], this does not appear to be the case. Alterations of voltage dependence are not in the same direction for Na^+ activation and inactivation parameters. Moreover, the block of Na^+ current caused by 1 mM octanol cannot be accounted for by the small change in voltage dependence observed.

Acknowledgements

We thank Drs. James Hall, Sidney Simon and J.W. Moore for helpful discussions, and Dr. Edward Lieberman for instructions in axial wire techniques as applied to crayfish giant axons. Thanks are also due to Sasi Kumar, Paula Lee and Zarin Karanjia for secretarial assistance. This work was supported by NIH grant NS 14144.

References

1 Hubbell, W.L. and McConnell, H.M. (1968) Proc. Nat. Acad. Sci. U.S.A. 61, 12—16
2 Machleidt, H., Roth, S. and Seeman, P. (1972) Biochim. Biophys. Acta 255, 178—189
3 Miller, K.W. (1975) in Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia (Fink, B.R., ed), pp. 341—351, Raven Press, New York
4 Ueda, I., Shieh, D.D. and Eyring, H. (1975) in Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia (Fink, B.R., ed), pp. 291—305, Raven, Press, New York
5 Eyring, H., Woodburg, J.W. and D'Arrigo, J.S. (1973) Anesthesiology 38, 415—424
6 Hsia, J.C. and Boggs, J.M. (1975) in Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia (Fink, B.R., ed.), pp. 327—338, Raven Press, New York
7 Bennett, P.B., Simon, S. and Katz, Y. (1975) in Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia (Fink, B.R., ed.), pp. 367—403, Raven Press, New York
8 Johnson, S.M. and Miller, K.W. (1970) Nature 228, 75—76
9 Trudell, J.R. and Cohen, E.N. (1975) in Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of Anesthesia (Fink, B.R., ed.), pp. 315—325, Raven Press, New York
10 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 328—334
11 Metcalfe, J.C., Seeman, P. and Burgen, A.S.V. (1968) Mol. Pharmacol. 4, 87—95
12 Bennett, P.B. and Hayward, A.J. (1967) Nature 213, 938—939
13 Shrivastav. B., Narahashi, T., Kitz, R.J. and Roberts, J.D. (1976) J. Pharmacol. Exp. Ther. 199, 179—188
14 Simon, S.A., Hall, J.E. and Bennett, P.B. (1977) Am. Soc. Anesthesiol. Annu. Meet. Abstr. 613
15 Armstrong, C.M. and Binstock, L. (1964) J. Gen. Physiol. 48, 265—277
16 Van Harreveld, A. (1936) Proc. Soc. Exp. Biol. Med. 34, 428—432
17 Lieberman, E.M. and Lane, T.G. (1976) Pflügers Arch. 366, 189—193
18 Shrager, P. (1974) J. Gen. Physiol. 64, 666—690
19 Cole, K.S. and Moore, J.W. (1960) J. Gen. Physiol. 44, 123—167
20 Hodgkin, A.L. and Huxley, A.F. (1952) J. Physiol. 116, 497—506
21 Hille, B. (1967) Dissertation number 68-9584, University Microfilms, Inc., Ann Arbor, Michigan
22 Swenson, R.P. and Oxford, G.S. (1980) in Progress in Anesthesiology, Vol. 2, Molecular Mechanisms of Anesthesia (Fink, B.R., ed.), Raven Press, New York, in the press
23 Shrager, P. (1977) J. Gen. Physiol. 69, 183—202
24 Swenson, R.P. (1979) Biophys. J. 25, 136a
25 Hille, B. (1977) J. Gen. Physiol. 69, 497—515
26 Schwartz, W., Palade, P.T. and Hille, B. (1977) Biophys. J. 20, 343—368
27 Cahalan, M. (1978) Biophys. J. 23, 285—311
28 Yeh, J.Z. and Narahashi, T. (1977) J. Gen. Physiol. 69, 293—323
29 Armstrong, C.M. and Bezanilla, F. (1974) J. Gen. Physiol. 63, 533—552